Development of a Regulatory Value for the Carcinogenic Potential of Isoprene via Inhalation Exposure

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ABSTRACT

Isoprene is the 2-methyl analogue of 1,3-butadiene, which is currently classified as a known human carcinogen. While isoprene is synthesized and used in the manufacturing of substances such as synthetic rubber, it is also produced naturally by plants, animals, and bacteria and is one of the main endogenous compounds found in human breath. Following the Texas Commission on Environmental Quality (TCEQ) Guidelines for the development of toxicity factors, a preliminary review and characterization of the carcinogenic potential of isoprene was conducted. Three key animal studies provided adequate data for the dose-response assessment of isoprene's carcinogenic potential. In order to determine URFs for study endpoints assuming exposure for 24h/d, 7d/wk, for a lifetime, the dose levels and numbers of animals at risk in the data sets were adjusted for differences between the exposure durations and times of response observation. The doses were adjusted to the constant lifetime environmental dose that is equivalent to the time-dependent doses in the studies, based on the multistage theory of carcinogenesis using the Armitage and Doll (1954) mathematical description of carcinogenesis with the number of stages being m = 1, 2, or 3. Similarly, the number of subjects at risk of developing the specified response by necropsy time in the study was adjusted to the equivalent number of animals at risk if the time to necropsy were equal to the nominal animal lifetime. The adjusted parameters were used to carry out 171 model fits. The EC_{10} for each endpoint was identified using the estimated multistage models, and from there a URF for each endpoint was calculated. Based on the TCEQ Guidelines, only malignant endpoints considered relevant to humans and showing a statistical significance were considered for the draft URF. The chosen draft URF was 9.1E-04 per ppm for liver carcinoma in a one stage carcinogenic process (m=1). From the draft URF, a draft air concentration corresponding to a 1E-05 excess cancer risk level is calculated to be 11 ppb.

CARCINOGENIC POTENTIAL

There are currently no human exposure studies available for isoprene; however, there are three chronic animal studies available that provide evidence of carcinogenicity in mice (Melnick et al. 1994 and Placke et al. 1996) and rats (Melnick et al. 1999). Increased incidences of neoplasms were observed in the lungs, liver, harderian gland, forestomach, hematopoitic system, and circulatory system in mice exposed to isoprene via inhalation. In rats, increased incidences of neoplasms were observed in the mammary gland, kidney, and testis. While there are currently no human exposure studies indicating that inhalation exposure to isoprene increases the risk of cancer, due to the formation of tumors at multiple sites in multiple animal species, isoprene has been classified by the National Toxicology Program and the International Agency for Research on Cancer as a potential human carcinogen. As such, it is the policy of the TCEQ to conduct a carcinogenic dose-response assessment for chemicals considered "likely to be carcinogenic to humans."

Agency	Classification	Basis
NTP ROC[a]	Reasonably anticipated to be a human carcinogen	Evidence of tumor formation at multiple organ sites in multiple species of experimental animals
IARC[b]	2B; Possibly carcinogenic to humans	Evidence of tumor formation at multiple organ sites in multiple species of experimental animals

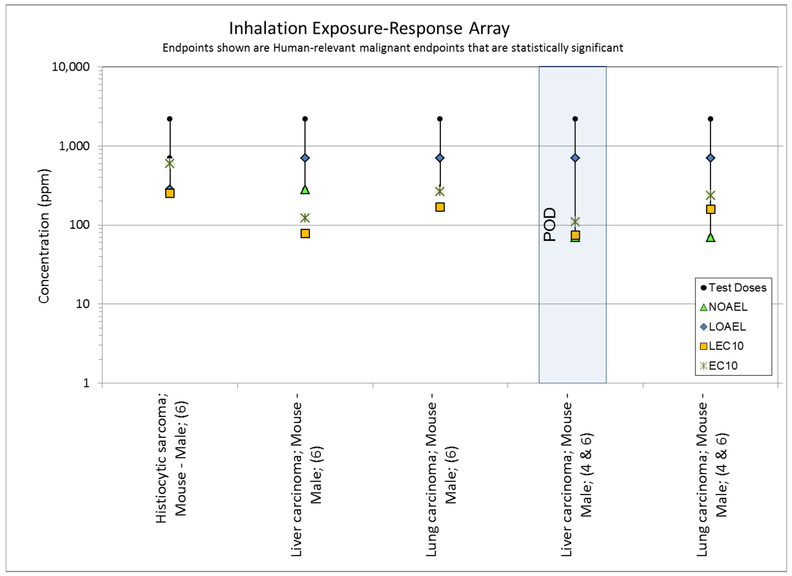
[a]National Toxicology Program's Report on Carcinogens
[b]International Agency for Research on Cancer

KEY STUDIES

Due to a lack of available human data, three animal studies were considered for the development of a chronic carcinogenic toxicity factor for isoprene. Due to the complexity of the available animal data, TCEQ hired a statistical expert to review and model the data, Sielken & Associates Consulting, Inc. The following studies were determined to be adequate for a dose-response analysis.

Study	Species	Sex	#/Group	Exposure (ppm)	Duration		
					h/d	d/wk	Total (wks)
Melnick et al. 1994	F344/N rats and $B6C3F_1$ mice	Male	40	70, 220, 700, 2,200, or 7,000	8	5	24 + 24 wks recovery
Melnick et al. 1999	F344/N rats	Male & female	50	220, 700, or 7,000	6	5	105
Placke et al. 1996	$B6C3F_1$ mice	Male	50	280	8	5	20
		Male	50	2,200	4	5	20
		Male	50	70, 140, or 2,200	8	5	40
		Male & female	50	10 or 70	8	5	80
		Male	50	280, 700, or 2,200	8	5	80
		Male	50	2,200	4	5	80

EXPOSURE-RESPONSE ARRAY



6 = Placke et al. 1996
4 & 6 = Combined Melnick et al. 1994 and Placke et al. 1996

MATHEMATICAL ADJUSTMENTS TO THE DATA

Dose Scale Adjustments

In order to evaluate the data using the multistage theory of carcinogenesis (the Armitage and Doll (1954) mathematical description of carcinogenesis as expressed by Crouch (1983), Crump and Howe (1984), and several others), the intermittent experimental doses in these key studies were transformed to equivalent doses and used to estimate EC_{10}s. The formula to carry this dose adjustment out is:

$$D = d\ x\ \left(\frac{n_{hrs}}{24}\right) x\ \left(\frac{n_{days}}{7}\right) x\ \frac{(T_e - a)^m - (T_e - b)^m}{T^m}$$

Where:
D = equivalent lifetime average daily dose
n_{hrs} = hours of exposure per day
T_e = total study duration, in weeks
a = time when exposure begins, in weeks
b = time when exposure ends, in weeks
d = experimental dose
n_{days} = days of exposure per week
T = time, in weeks, corresponding to the end of a normal lifetime; 104 weeks for a 2-year lifetime in mice and rats
m = cancer stage, m = 1, 2, or 3

Number of Subjects Adjustment

Once the doses have been adjusted, a potential inequality will be present in the dose-response modeling if the end of a study (T_{end}) is not equal to the end of a nominal lifetime (T). In that case, the number of subjects at risk of developing the specified response by the end of a nominal lifetime in the dose-response modeling needs to be adjusted. For this adjustment, the following equation was utilized if $T_{end} \leq T$:

$$Adjusted\ n_{at\ risk,i} = n_{resp,i} + \left(n_{at\ risk,i} - n_{resp,i}\right) x\ \left(\frac{T_{end}}{T}\right)^m$$

If $T_{end} > T$, the following equation was utilized:

$$Adjusted\ n_{at\ risk,i} = n_{resp,i}\ x\ \left(\frac{T_{end}}{T}\right)^m + \left(n_{at\ risk,i} - n_{resp,i}\right)$$

Where:
$n_{at\ risk,\ i}$ = the number of subjects in the i^{th} dose group at the start of the study
$n_{resp,\ i}$ = the number of subjects that are observed to have the specified response by the end of the study
T_{end} = end of the study
T = end of a nominal lifetime
m = cancer stage, m = 1, 2, or 3

DETERMINATION OF THE POD

The TCEQ Guidelines to Develop Toxicity Factors (TCEQ 2012) was followed in determining the appropriate Point of Departure (POD). Sielken et al. (2012) carried out a total of 171 dose-response model fits, using a total of 57 endpoints (i.e., 57 combinations of study, species, gender, organ, and severity), with the multistage-cancer model. Since the ultimate endpoint for carcinogenic characterization is cancer, consideration of the POD was only given to malignant endpoints that are considered relevant to humans and that were found to be statistically significant in the key studies. The EC_{10} and LEC_{10}, estimated for the relevant endpoints, are as follows:

Study	Endpoint	Species	EC_{10} (ppm)			LEC_{10} (ppm)		
			m=1	m=2	m=3	m=1	m=2	m=3
Placke et al. 1996	Histiocytic Sarcoma	B6C3F1 mice	600.67	525.59	446.69	252.70	262.40	242.60
Placke et al. 1996	Liver Carcinoma	B6C3F1 mice	122.96	131.36	125.58	78.50	87.76	86.07
Placke et al. 1996	Lung Carcinoma	B6C3F1 mice	263.11	313.39	310.26	168.80	203.20	203.50
Combined Melnick et al. 1994 & Placke et al. 1996	Liver Carcinoma	B6C3F1 mice	**109.85**	118.53	129.86	**74.68**	83.15	83.17
Combined Melnick et al. 1994 & Placke et al. 1996	Lung Carcinoma	B6C3F1 mice	235.00	278.11	299.60	158.90	186.70	189.00

The relevant endpoint with the lowest estimated value was chosen as the critical endpoint. The EC_{10} represents the best estimate lifetime excess cancer risk resulting from continuous exposure to isoprene, whereas the LEC_{10} represents the lower bound of that estimate. The EC_{10} for cancer stage m=1 was chosen as the POD for the following reasons:

⇒ The EC_{10} represents the best estimate of the most sensitive species and sex tested (male mice)

⇒ The most sensitive endpoint was used, rather than combining all endpoints, which is more conservative

	Central Tendency for m=1 (ppm)			Ratio of Central Tendency to Chosen EC_{10}[a]		
	Geometric Mean	Average	Median	Geometric Mean	Average	Median
EC_{10}	218.82	266.32	235.00	1.99	2.42	2.14
LEC_{10}	131.78	146.72	158.90	1.20	1.34	1.45

[a]Chosen EC_{10} = 109.85 ppm

A conservative default dosimetric adjustment factor of 1 was applied because the blood:gas partition coefficient for mice is greater than that for humans (2.04 and 0.75, respectively).

DRAFT TOXICITY FACTOR

A draft Unit Risk Factor (URF) and isoprene air concentration at 1 in 100,000 excess cancer risk were calculated using the above POD. Without strong evidence of a non-linear MOA, the default procedure is to use a linear approach to this calculation. To determine the best estimate lifetime excess cancer risk resulting from continuous exposure to isoprene at 1 $\mu g/m^3$, the following equation is used:

$$URF = \frac{0.10}{EC_{10}}$$

The 10^{-5} risk air concentration is then calculated based on the URF using the following equation:

$$10^{-5}\ risk\ air\ concentration = \frac{1\ x\ 10^{-5}}{URF}$$

The DRAFT calculated URF and air concentration corresponding to 1 in 100,000 excess cancer risk are:

Study	Endpoint	DRAFT URF (risk per ppb)	DRAFT 10^{-5} Risk Air Concentration (ppb)
Combined Melnick et al. 1994 & Placke et al. 1996	Liver carcinoma	9.1E-07	11

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